Association of Serum Afamin Concentrations With Kidney Failure in Patients With CKD: Findings From the German CKD Cohort Study

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Rationale & Objective: Afamin is a vitamin E-binding glycoprotein primarily expressed in the liver and kidney. This study investigated whether serum afamin concentrations are associated with kidney function and incident kidney failure.

Study Design: Prospective cohort study with 6.5 years follow-up.

Setting & Participants: 5,041 White patients enrolled in the German Chronic Kidney Disease (GCKD) study with measured afamin concentrations and either an estimated glomerular filtration rate (eGFR) of 30-60 mL/min/1.73 m^2 or an eGFR > 60 mL/min/1.73 m^2 with a urinary albumin-creatinine ratio (UACR) of ≥300 mg/g at study entry.

Exposure: Serum afamin concentrations (mg/L).

Outcome: Incident kidney failure (initiation of kidney replacement therapy or kidney-related death).

Analytical Approach: Generalized linear regression and quantile regression models fit to investigate the association of afamin concentrations with eGFR and UACR. Adjusted Cox regression analysis to examine the association of afamin concentrations with incident kidney failure.

Results: The mean ± SD afamin concentration at study entry was 73.2 ± 17.6 mg/L. Higher afamin concentrations were associated with better kidney function with a 2.60 mL/min/1.73 m^2 higher eGFR (95% CI, 2.30-2.89) and a 5.97 mg/g lower UACR (95% CI, 3.04-8.90) for each 10 mg/L higher level of afamin concentration in adjusted analysis. During the follow-up period, each 10 mg/L higher level of afamin concentration was associated with a 14% lower risk of kidney failure (HR, 0.86 [95%CI, 0.81-0.92], $P < 0.001$).

Limitations: Residual confounding, and potential limited generalizability to non-White populations and people with mild stages of chronic kidney disease (CKD) or no CKD.

Conclusions: Higher serum afamin concentrations appear to be associated with a higher eGFR, less albuminuria, and a lower risk for future kidney failure in patients with CKD.

Visual Abstract online

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More than 10% of the adult population worldwide suffers from chronic kidney disease (CKD). Roughly, 1.2 million deaths and 28 million years of life lost can be attributed to CKD each year.[1] Moreover, CKD is one of the fastest growing causes of death and predicted to become the fifth leading cause of death in 2040. A decrease in glomerular filtration rate (GFR) and an increase in urinary albumin-creatinine ratio (UACR) are key parameters describing overall kidney function and an increased risk for CKD progression and death, respectively.[2] Importantly, novel treatment opportunities have arisen or are under development that may retard the progression of CKD and postpone the onset of kidney failure.[3] A rational and efficient use of these therapies requires identification of CKD patients with high risk for progression.

Afamin, a human serum vitamin E–binding glycoprotein, was first described in 1994 by Lichenstein et al[4] as the fourth member of the human albumin gene family including albumin, α-fetoprotein, and vitamin D-binding protein. It has a molecular mass of 87 kD with 15% carbohydrate content and 55% amino acid sequence similarity to albumin.[4-6] Substantial amounts of afamin are present in serum, and, to a lesser extent, in extravascular fluids such as cerebrospinal, follicular, and seminal fluid.[7] The liver is the major site of afamin gene expression[4] with lower expression levels in the brain, testes, ovaries, and kidney[7] (www.proteinatlas.org/ENSG00000079557-AFM).

As yet, little is known about the (patho)-physiological functions of afamin.[7-9] Several vitamin E–binding sites of human afamin have been detected with a radioligand assay assuming binding affinity of afamin for both α- and γ-tocopherol.[10] Furthermore, Naschberger et al[11] reported the crystal structure of afamin suggesting a structural basis of afamin for Wnt solubilization with implications for embryonic intercellular signaling. Transgenic mice overexpressing the human afamin gene developed increased body weight, lipids, and glucose concentrations. In 2 large pooled analyses of up to 20,000 study participants we showed that afamin concentrations are associated with the prevalence and incidence of metabolic syndrome and diabetes mellitus.[8,9]

One small case control[12] and 2 proteomics studies[13,14] measuring afamin in urine samples described afamin as a potential marker of kidney disease. Based on these findings, we investigated whether serum afamin concentrations are associated with kidney function and future risk

for kidney failure. We measured serum afamin in the German Chronic Kidney Disease (GCKD) study, a large, prospective cohort study of 5,041 patients with CKD.[15]

Methods

German Chronic Kidney Disease (GCKD) Study

The GCKD study is a multicenter national prospective cohort study with ongoing follow-up investigations. Between the years 2010 and 2012, altogether 5,217 White patients with CKD were recruited. Further details on the design and characteristics of the study have already been published.[15-17] Briefly, the study aimed to enroll patients with mild to severe CKD mostly in stage G3 under regular care by nephrologists. The main inclusion criteria were an estimated glomerular filtration rate (eGFR) of 30-60 mL/min/1.73 m^2 (KDIGO stage G3, A1-3) or an eGFR of >60 mL/min/1.73 m^2 in the presence of overt proteinuria as defined by a UACR of ≥300 mg/g or equal (KDIGO stage G1-2, A3). The defined exclusion criteria comprised non-White ethnicity, solid organ or bone marrow transplantation, active malignancy within 24 months before screening, heart failure of New York Heart Association stage IV, and legal attendance or inability to provide consent. At baseline, blood samples were collected by trained personnel, processed, and sent on dry ice to a central biobank where routine laboratory parameters were measured centrally.[15] The eGFR was calculated using the CKD-EPI equation.[18]

Each participant provided written informed consent, and all analyses were performed in accordance with approved guidelines and the Declaration of Helsinki. The study was approved by the ethics committees of each regional center and registered in the national registry for clinical studies (DRKS 00003971). Data are collected and managed using Askimed (https://www.askimed.com) as a cloud-based web platform.

Patients are followed on a yearly basis by trained personnel collecting data on hospitalizations, outcome events, and medical history using a structured interview. Any hospital discharge reports are collected from the treating physicians and/or hospitals. End points are continuously extracted from these reports by an end point adjudication committee.

Definition of Outcome

The end point investigated in this study was kidney failure during the first 6.5 years of follow-up evaluation based on data export from October 8, 2020. Kidney failure was marked as the start of any form of dialysis, or kidney transplantation, or kidney-related death (death due to forgoing of dialysis).

Measurement of Afamin Serum Concentrations

Serum afamin concentrations were measured at the Institute of Genetic Epidemiology of the Medical University of Innsbruck with a custom-made double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) using an affinity-purified biotinylated polyclonal anti-afamin antibody for coating 96-well streptavidin-bound microtiter plates and a peroxidase-conjugated monoclonal antibody (N13) for detection (MicroCoat Biotechnologie GmbH). The within-run and between-run coefficients of variation were 3.3% and 6.2%, respectively.[9]

Statistical Analysis

Characteristics of the GCKD study participants at the baseline investigation were described by quartiles of afamin concentrations. Distribution of afamin concentration was plotted based on a histogram. Linear regression analysis was applied to identify variables independently associated with afamin concentrations. Generalized linear regression models were fit to investigate the association of afamin concentrations with continuous eGFR. Quantile regression analysis was applied for continuous UACR due to its non-normal distribution. Within this analysis, all parameters are estimated at the 0.50 quantile (= median).

Cox proportional hazards regression and respective hazard ratios (HR) including 95% confidence intervals (95%CI) were calculated for the end point kidney failure. For this end point all deaths from other causes were treated as censored observations. Time to adverse kidney events was defined from study entry to the particular first event. Linearity of afamin on adverse kidney events was tested by a penalized, age, sex, eGFR, and UACR adjusted regression spline approach.[19] The proportional hazards assumption was tested by χ^2-test based on Schoenfeld residuals. Furthermore, subdistribution hazard ratios (SHR) out of competing risks survival regression were calculated defining all other causes of death as competing events.[20]

The adjustment of the data in the various models was performed based on clinical reasons and by taking differences of variables between quartiles of afamin into account. In addition, a risk score developed by Zacharias et al[21] was taken to further test whether afamin is independently associated with the outcome in further Cox regression analyses. The variables for adjustment are given in the results section and in the various table legends. An internal validation of Cox regression analyses results was done by a bootstrapping approach based on resampling (1,000 bootstrap samples) with replacement from the original sample and using 95% confidence intervals based on percentiles.[22]

The continuous prospective net reclassification index (NRI) was calculated for a follow-up time of 2 and 6.5 years, respectively, to evaluate whether afamin concentrations contribute to a better risk prediction of kidney failure. Details are provided in the supplementary material (additional analyses, Item S1). The natural logarithm (ln) was applied to log-transform the variables UACR, high-sensitivity C-reactive protein (hs-CRP), and triglycerides due to their skewed distribution. Estimates were shown for an increment of 10 mg/L in afamin concentrations as well as for quartile groups of afamin (quartile 1 used as reference).

Table 1. Baseline Characteristics of German Chronic Kidney Disease (GCKD) Study Patients Stratified by Quartiles of Afamin

	Afamin Quartiles			
	Quartile 1 (n = 1,264)	Quartile 2 (n = 1,262)	Quartile 3 (n = 1,257)	Quartile 4 (n = 1,258)
Afamin range, mg/L	10.6-60.5	60.5-72.1	72.2-84.4	84.4-151.9
Afamin	52.1 ± 6.7	66.5 ± 3.2	78.0 ± 3.5	96.5 ± 10.6
No. of patients	1,264	1,262	1,257	1,258
Age, y	58 ± 13	59 ± 12	61 ± 11	62 ± 11
Female gender	414 (33%)	529 (42%)	520 (41%)	544 (43%)
BMI, kg/m^2	26.9 ± 5.0	29.2 ± 5.7	30.8 ± 5.6	32.4 ± 6.0
Smoker or ex-smoker	734 (58%)	711 (57%)	739 (59%)	786 (63%)
Diabetes	353 (28%)	375 (30%)	468 (37%)	585 (47%)
Hypertension	1,203 (95%)	1,202 (95%)	1,224 (97%)	1,223 (97%)
SBP, mm Hg	139 ± 21	138 ± 20	140 ± 20	141 ± 20
DBP, mm Hg	78 ± 12	79 ± 12	79 ± 12	80 ± 12
Cardiovascular disease	299 (24%)	327 (26%)	333 (27%)	330 (26%)
Kidney parameters				
eGFR, mL/min/1.73 m^2	47 ± 18	49 ± 18	50 ± 18	52 ± 19
UACR, mg/g	91 [14-525]	55 [11-402]	37 [8-313]	35 [7-294]
eGFR ≥ 60 and UACR ≥ 300	86 (7%)	96 (8%)	94 (8%)	121 (10%)
Cystatin C, mg/L	1.6 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.5
Serum urea, mg/dL	66.4 ± 29.2	61.4 ± 26.4	58.9 ± 23.8	56.4 ± 23.5
Serum creatinine, mg/ dL	1.6 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.4 ± 0.5
Medications				
Antihypertensive	1,164 (92%)	1,168 (93%)	1,197 (95%)	1,205 (96%)
Diabetes	306 (24%)	298 (24%)	384 (31%)	424 (34%)
Statins	512 (41%)	593 (47%)	632 (50%)	656 (52%)
Triglyceride-lowering agents	33 (3%)	35 (3%)	57 (5%)	113 (9%)
Blood proteins				
Serum albumin, g/L	37.8 ± 4.5	38.2 ± 4.8	38.6 ± 4.0	39.0 ± 4.2
Hemoglobin, g/dL	13.2 ± 1.6	13.4 ± 1.6	13.8 ± 1.7	14.1 ± 1.6
hs-CRP, mg/L	1.99 [0.83-4.88]	2.22 [0.95- 5.01]	2.36 [1.07-5.20]	2.42 [1.25-4.88]
Parameters of lipid metabolism				
Total cholesterol, mg/dL	203 ± 55	212 ± 51	214 ± 54	216 ± 50
LDL cholesterol, mg/dL	117 ± 46	120 ± 43	120 ± 44	117 ± 41
HDL cholesterol, mg/dL	55 ± 18	54 ± 18	50 ± 17	50 ± 18
Triglycerides, mg/dL	128 [94-180]	157 [112-215]	186 [135-257]	216 [152-311]

Values are provided as mean ± SD and median [IQR, Q1-Q3] where appropriate, or as number of patients, n (%) if not indicated otherwise. In the total group (n = 5,041), for all variables displayed, number of missing values were ≤3.0%. BMI was corrected for amputation. Hypertension was defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg, and/or receiving antihypertensive treatment. Cardiovascular disease was defined as myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, stroke, interventions at the carotid arteries. The eGFR was calculated according to the CKD-EPI equation. The hs-CRP and urinary albumin values that were below the lower detection limit (LOD) were replaced by LOD/√2. UACR was calculated according to the following equation: Albumin in urine (mg/L) × 100 / Creatinine in urine (mg/dL) and is given in mg/g. Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure; UACR, urinary albumin-creatinine ratio.

Statistical analysis was performed using SPSS for Windows, version 29.0 (IBM Corp.), and R for Windows, version 4.3.1 (R Project). For all analyses a 2-sided test $P < 0.05$ was considered statistically significant.

Results

Baseline Analysis

The main analyses of this study are based on 5,041 of 5,217 GCKD study patients with complete baseline data for age, sex, eGFR, UACR, and afamin. The mean ± SD concentration of afamin was 73.2 ± 17.6 mg/L. Clinical characteristics of patients at the baseline investigation are displayed according to quartiles of afamin concentrations (Table 1). A histogram plot revealed a normal distribution of afamin concentrations (Fig S1).

The association of clinical and laboratory variables with afamin concentrations are shown in Table 2. Triglycerides, BMI, eGFR, age, female sex, smoking status, diabetes, and diastolic blood pressure were independently and positively associated with afamin concentrations, whereas hs-CRP, UACR, and systolic blood pressure showed a negative association (Table 2). Because reduced afamin concentrations could reflect an increased renal clearance, we tested for effect modification by UACR. However, an interaction term of UACR * eGFR in Table 2 did not reveal any evidence ($P = 0.4$).

Table 2. Linear Regression Analysis Investigating the Association of Clinical Parameters With Afamin Concentrations

	β Estimate	SE	*t* Statistic	*P* Value
Triglycerides, 10% increase[a]	1.085	0.042	26.085	<0.001
BMI, 1 kg/m^2	0.797	0.041	19.524	<0.001
eGFR, 10 mL/min/1.73 m^2	2.179	0.127	17.191	<0.001
Age, 10 y	2.367	0.227	10.448	<0.001
Female sex	4.607	0.459	10.038	<0.001
Diastolic blood pressure, 10 mm Hg	1.689	0.239	7.057	<0.001
hs-CRP, 10% increase[a]	−0.136	0.020	−6.889	<0.001
UACR, 10% increase[a]	−0.058	0.011	−5.413	<0.001
Systolic blood pressure, 10 mm Hg	−0.472	0.140	−3.365	<0.001
Current and ex-smoker	1.298	0.449	2.890	0.004
Diabetes	1.366	0.501	2.726	0.006
Statin use	0.909	0.448	2.031	0.04

All variables listed are included in the analysis at the same time. Estimates for continuous variables are provided based on clinically relevant increments. Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; hs-CRP, high- sensitivity C-reactive protein; SE, standard error; UACR, urine albumin-creatinine ratio.
[a]Beta estimates and standard errors of highly skewed clinical parameters are based on ln-transformed values. Increment is provided for a 10%-increase of the ln-transformed variables.

Association of Kidney Function Parameters With Serum Afamin Concentrations

A heat map plot based on combined categories of eGFR and UACR shows mean unadjusted afamin concentrations according to the Kidney Disease Improving Global Outcomes (KDIGO) stratification criteria (Fig 1). Mean afamin concentrations were higher in individuals with better kidney function (higher eGFR and lower UACR). When we compared those with (n = 1,414) and without overt proteinuria (n = 3,627), the percentage of patients with diabetic nephropathy was comparable between the 2 groups (26.4% vs 26.7%, P = 0.79). However, those with overt proteinuria had significantly lower hemoglobin A_{1c} (HbA_{1c}) values (median 5.9% vs 6.0%, P < 0.001).

Results of a fully adjusted linear regression model on continuous eGFR revealed a 2.60 mL/min/1.73 m^2 ([95% CI, 2.30-2.89], P < 0.001) higher eGFR with each 10 mg/L increment of afamin. This translates into a 5% relative increase as compared with the overall mean of predicted eGFR values of the total group taken as reference (Table 3). The linearity of the association was underscored when the analyses were based on afamin quartiles resulting in 10%, 18%, and 27% relative higher eGFR values in quartiles 2, 3, and 4 when compared with the marginal mean of quartile 1

GCKD study

eGFR (mL/min/1.73 m²)	Urine albumin-creatinine ratio (UACR) (mg/g)				
	< 30	30 - 299	300 - 2220	> 2220	N total
>=60	77.4 ± 16.4 (n=395)	75.3 ± 17.4 (n=261)	75.8 ±18.2 (n=339)	78.1 ±18.4 (n=58)	1053
45-59	75.8 ± 17.1 (n=859)	72.7 ± 17.6 (n=458)	71.8 ± 18.2 (n=285)	76.3 ± 18.5 (n=53)	1655
30-44	74.1 ± 17.6 (n=762)	71.0 ± 16.8 (n=591)	68.0 ±16.4 (n=422)	69.9 ±18.4 (n=73)	1848
<30	71.9 ± 15.5 (n=139)	70.8 ± 19.8 (n=162)	66.6 ±16.8 (n=139)	65.3 ± 18.1 (n=45)	485
N total	2155	1472	1185	229	5041

Afamin (mg/L)	≤ 71.0	> 71.0 - 73.0	> 73.0 - 75.0	> 75.0

Figure 1. Mean ± SD afamin concentrations and number of patients stratified by eGFR and UACR risk categories (including nephrotic range UACR > 2,220 mg/g) according to KDIGO guidelines in the GCKD Study. Mean afamin concentrations were higher in individuals with better kidney function (higher eGFR and lower UACR). Increasing concentrations of afamin are displayed with cell backgrounds with lighter blue background colors (change in color per 2 mg/L increment of afamin concentrations). Note: numbers of patients do not add up to the total number from GCKD with available afamin values due to missing values for eGFR and UACR. Abbreviations: eGFR, estimated glomerular filtration rate; GCKD, German Chronic Kidney Disease; UACR, urinary albumin-creatinine ratio.

Table 3. Linear and Quantile Regression Analysis Investigating the Association of Afamin Concentrations on Continuous eGFR and UACR

	β Estimate (95% CI)	*P* Value	Comparisons
Linear Regression, eGFR			**Relative Increase Compared to Mean[a]**
Calculations per 10 mg/L increment of afamin concentrations			
Adjusted analysis[b]	2.60 (2.30 to 2.89)	<0.001	~ 5%
Calculations per quartile of afamin concentrations			
Quartile 1 (reference)			
Quartile 2	4.46 (3.15 to 5.77)	<0.001	~ 10%
Quartile 3	7.72 (6.35 to 9.09)	<0.001	~ 18%
Quartile 4	11.60 (10.16 to 13.05)	<0.001	~ 27%
Quantile Regression, UACR			**Relative Decrease Compared to Median[c]**
Calculations per 10 mg/L increment of afamin concentrations			
Adjusted analysis[b]	−5.97 (−8.90 to −3.04)	<0.001	~ 7%
Calculations per quartile of afamin concentrations			
Quartile 1 (reference)			
Quartile 2	−17.25 (−29.79 to −4.71)	0.007	~ 15%
Quartile 3	−30.46 (−43.63 to −17.29)	<0.001	~ 27%
Quartile 4	−31.65 (−45.72 to −17.57)	<0.001	~ 28%

Abbreviations: eGFR, estimated glomerular filtration rate; UACR, urinary albumin-creatinine ratio.
[a]Either overall mean of predicted eGFR values of respective models was taken as reference or marginal mean from quartile 1 where appropriate.
[b]Adjusted for age, sex, UACR or eGFR where appropriate, ln-triglycerides, ln-high sensitivity C reactive protein, systolic and diastolic blood pressure, smoking status, diabetes, body mass index, statin use.
[c]Either overall median of predicted UACR values of respective models was taken as reference or marginal median from quartile 1 where appropriate.

($P < 0.001$), respectively (estimates and 95% CI are provided in Table 3).

Similar results were found with UACR as dependent variable. With each increment of afamin concentrations by 10 mg/L, UACR was significantly lower in the fully adjusted quantile regression model ($\beta = -5.97$ [95% CI, −8.90 to −3.04], $P < 0.001$). This was equivalent to a 7% relative decrease compared with the overall median of predicted UACR values of the total group. The UACR concentration was lowest in the fourth quartile of afamin concentrations versus the first quartile ($\beta = -31.65$ [95% CI, −45.72 to −17.57], $P < 0.001$). This translates to a 28% relative decrease compared with the marginal median of UACR in the quartile 1 group (Table 3).

Association of Serum Afamin Concentrations With Incident Kidney Failure

During a median follow-up time of 6.5 years, 475 out of 5,041 patients (9.4%) experienced a progression toward kidney failure requiring dialysis treatment (n = 394) and/or kidney transplantation (n = 64) or died due to forgoing of dialysis (n = 17). We additionally tested the linearity of afamin with kidney failure by nonlinear P spline analyses. We observed a linear effect that was highly significant ($P < 0.001$) whereas the nonlinear effect was not ($P = 0.40$). The corresponding spline plot is shown in Figure S2.

There was no indication for violation of the proportional hazards assumption. Table 4 summarizes the results from Cox regression analyses. Each increment of afamin by 10 mg/L was associated with a 12% lower risk for kidney failure during the observation period in the age, sex, and kidney function adjusted model 1 (HR, 0.88 [95% CI, 0.83-0.93], $P < 0.001$). This association remained significant in the fully adjusted model 3 additionally considering triglycerides, hs-CRP, systolic and diastolic blood pressure, smoking status, diabetes, BMI, and statin use. This translates into a 14% lower risk for kidney failure (HR, 0.86 [95% CI, 0.81-0.92], $P < 0.001$). Comparable results (10% lower risk for kidney failure) were observed when afamin was added to the 6-variable equation developed by Zacharias et al[21] which includes UACR, serum creatinine, serum albumin, hemoglobin, urea, and cystatin C (HR, 0.90 [95% CI, 0.85-0.95], $P < 0.001$).

An analysis according to quartiles of afamin was in line with the results of the continuous analysis, reaching statistical significance in all 3 quartiles and adjustment models (Table 4). The subdistribution hazard ratios were only very slightly attenuated (treating nonrenal death as a competing risk event) (Table S1) as compared with the hazard ratios shown in Table 4.

When we stratified patients into those in stage G1-G3a versus stage G3b and higher, the estimates were very similar to those shown in Table 4 for all 3 models (Table S2).

Exploratory Analysis of Risk Prediction Properties of Serum Afamin Concentrations for Kidney Failure

Finally, we calculated the prospective continuous net reclassification index (NRI) to evaluate whether afamin

Table 4. Association of Afamin With Kidney Failure During the Prospective Follow-up

	No. at Risk[a]	No. of Events	Event Rate[b]	HR (95% CI)	*P*
Calculations per 10mg/L Increment of Afamin Concentrations					
Model 1	5,041	475	15.8	0.88 (0.83-0.93)	<0.001
Model 2	4,938	468	15.9	0.90 (0.85-0.95)	<0.001
Model 3	4,943	462	15.7	0.86 (0.81-0.92)	<0.001
Calculations per 10mg/L Increment of Afamin Concentrations					
Model 1[c]					
Quartile 1	1,264	194	27.0	1.00	
Quartile 2	1,262	125	16.5	0.73 (0.58-0.91)	0.006
Quartile 3	1,257	86	11.4	0.61 (0.47-0.79)	<0.001
Quartile 4	1,258	70	9.1	0.59 (0.45-0.79)	<0.001
Model 2[d]					
Quartile 1	1,240	193	27.4	1.00	
Quartile 2	1,240	122	16.4	0.73 (0.58-0.92)	0.007
Quartile 3	1,238	84	11.3	0.60 (0.46-0.77)	<0.001
Quartile 4	1,220	69	9.2	0.61 (0.46-0.81)	<0.001
Model 3[e]					
Quartile 1	1,238	189	26.8	1.00	
Quartile 2	1,240	122	16.4	0.71 (0.56-0.90)	0.005
Quartile 3	1,233	84	11.4	0.58 (0.44-0.76)	<0.001
Quartile 4	1,232	67	8.8	0.54 (0.39-0.74)	<0.001

Abbreviation: HR, hazard ratio.
[a]The number at risk depends on the number of missing values in the various models: model 1: no missing values; model 2: 103 missing; model 3: 98 missing.
[b]Event rate per 1,000 person-year follow-up.
[c]Model 1: Adjusted for age, sex, estimated glomerular filtration rate, ln-urinary albumin-creatinine ratio (equals the risk equation developed by Tangri et al).[23]
[d]Model 2: Adjusted for ln-urine albumin-creatinine ratio, serum creatinine, serum albumin, hemoglobin, urea, and cystatin C (equals the risk equation developed by Zacharias et al).[21]
[e]Model 3: As model 1 plus ln-triglycerides, ln-high-sensitivity C-reactive protein, systolic and diastolic blood pressure, smoking status, diabetes, body mass index, and statin use.

adds significantly to risk prediction accuracy in individuals who progressed to kidney failure during the follow-up period (cases). After a follow-up of 2 years, the addition of afamin resulted in a significant improvement of 33% for kidney failure in cases when added to a model including parameters of the established 4-variable kidney failure risk equation developed by Tangri et al[23] (age, sex, eGFR, and UACR: NRI, 0.33 [95% CI, 0.07-0.54]) (Table 5). This improvement increased to 36% when clinical variables of the 6-variable risk equation developed by Zacharias et al[21] including UACR, serum creatinine, serum albumin, hemoglobin, urea, and cystatin C were included in the model (NRI, 0.36 [95% CI, 0.14-0.54]) (Table 5). After the median follow-up time of 6.5 years, afamin still significantly improved risk prediction in cases (NRI for both formulas 0.19, [95% CI, 0.12-0.24], and 0.17 [95% CI, 0.10-0.24], respectively). For both follow-up time periods (2 years and 6.5 years), the risk prediction improvement for controls was less pronounced with 4.3% to 9.6% which barely missed significance (Table 5).

Additional Analyses

The estimates of afamin remained perfectly stable based on a bootstrapping approach using variables included in the 3

Table 5. Gain in Risk Probability Prediction for Kidney Failure Comparing a Model Based on the Established 4-Variable Kidney Failure Risk Equation and a New 6-Variable Risk Equation With Models Additionally Including Afamin

	Overall NRI (95% CI)	NRI Cases (95% CI)	NRI Controls (95% CI)
Gain in 2-Year Risk Probability Prediction			
Model 1: Tangri equation + afamin[a]	0.391 (0.085 to 0.650)	0.330 (0.071 to 0.541)	0.061 (−0.003 to 0.135)
Model 2: Zacharias equation + afamin[b]	0.456 (0.146 to 0.677)	0.360 (0.137 to 0.541)	0.096 (−0.004 to 0.212)
Gain in 6.5-Year Risk Probability Prediction			
Model 1: Tangri equation + afamin[a]	0.231 (0.086 to 0.345)	0.188 (0.115 to 0.240)	0.043 (−0.026 to 0.114)
Model 2: Zacharias equation + afamin[b]	0.247 (0.104 to 0.341)	0.170 (0.098 to 0.237)	0.077 (−0.017 to 0.136)

NRI was considered significant when the 95%CI determined empirically across 100 subsampling runs excluded zero. Abbreviations: GCKD study, German Chronic Kidney Disease study; NRI, net reclassification index.
[a]Model 1: 4-variable kidney failure risk equation (developed by Tangri et al)[23] (including age, sex, ln-urinary albumin-creatinine ratio, estimated glomerular filtration rate) plus afamin.
[b]Model 2: 6-variable risk equation (developed by Zacharias et al)[21] (including ln-urinary albumin-creatinine ratio, serum creatinine, serum albumin, hemoglobin, urea, and cystatin C) plus afamin.

regression models of Table 4: model 1 (HR, 0.88 [95% CI, 0.83-0.94], $P < 0.001$), model 2 (HR, 0.90 [95% CI, 0.84-0.96], $P = 0.005$), and model 3 (HR, 0.86 [95% CI, 0.80-0.92], $P < 0.001$), respectively.

The association between afamin and kidney failure was observed in patients with (HR, 0.90 [95% CI, 0.82-1.00], $P < 0.05$) and without diabetes mellitus (HR, 0.84 [95% CI, 0.77-0.92], $P < 0.001$) and an interaction term was not significant ($P = 0.6$). An adjustment for antihypertensive medication and HbA_{1c} did not change the main results.

For sake of completeness, we applied 2 risk categorizations for progression to kidney failure proposed by Tangri et al.[23] Afamin still showed a gain in risk prediction accuracy specifically for cases (Item S1).

Discussion

The main results of this study at hand are (1) mean afamin concentrations were higher in individuals with better kidney function (higher eGFR and lower UACR) independent of other parameters influencing kidney function; (2) higher afamin concentrations at baseline were independently associated with a lower risk for kidney failure during a follow-up time of 6.5 years; (3) in an exploratory analysis afamin added improved risk prediction accuracy of individuals who developed kidney failure in a 2- and 6.5-year risk prediction model when added to the established 4-variable kidney failure risk equation developed by Tangri et al[23] as well as the recently developed 6-variable risk equation by Zacharias et al.[21]

Because afamin is a member of the human albumin gene family and has 55% amino acid sequence similarity to albumin,[4,5] it could be speculated that afamin has physiological properties comparable to those of albumin: UACR is known to be associated with an increased risk for CKD progression, kidney failure, and death.[2] In patients with IgA nephropathy an association of low serum albumin and kidney failure has been described that was attributed to the antioxidative function of albumin.[24] In albumin-pretreated mouse mesangial and kidney cells compared with γ-globulin-pretreated cells intracellular reactive oxygen species (ROS) and thus mitochondrial injury were significantly reduced.[24] Whether this mechanism could also provide an explanation for the possible antioxidative properties of afamin in kidney disease remains to be elucidated. Afamin seems to have binding properties for 2 major forms of antioxidative vitamin E, α-tocopherol and γ-tocopherol.[10] However, the antioxidative function of vitamin E is not yet clarified (reviewed in Brigelius-Flohé et al[25]).

In line with its potential vitamin E and lipid transporter properties, afamin was thought to be a transporter of hydrophobic molecules, and its similarity to human serum albumin might be of possible relevance for a role as a general drug transporter.[10,11] Further binding partners or ligands of afamin might be Wnt signaling proteins. Naschberger et al[11] described a potential physical association of afamin and Wnt proteins based on structural data. Whether this translates into a physiological functionality remains to be determined. Diseases such as diabetes mellitus and kidney disease have been linked to the canonical Wnt pathway. Moreover, Wnt activation was found to be of relevance in mice models of diabetic nephropathy.[26-28] However, it is important to state that the physiological function of Wnt proteins in diseases like CKD or diabetes might be different to that of afamin.

Interestingly, in our current study we found that low serum concentrations of afamin are associated with a greater risk for incident kidney failure, independent of serum albumin concentrations. Moreover, in the GCKD study, the correlation of afamin and serum albumin is rather small ($r = 0.106$, $P < 0.001$, adjusted for age, sex, eGFR, and UACR). Only 7% of the variation of afamin concentrations can be explained by serum albumin when adjusted for the same variables. The same holds true for Cox regression based on the Zacharias equation, where afamin was significantly associated with kidney failure independent of serum albumin and UACR. Thus, the association of afamin with kidney failure seems to be independent of albumin and might most likely be explained by another mechanism.

Afamin was so far discussed to be related to kidney disease by only 2 small ELISA-validated proteomics studies[13,14] and 1 case-control study[12] in urine in 247 controls and 129 patients with glomerulonephritis. Afamin was suggested as a marker for glomerular barrier function due to its positive correlation with UACR. No correlation was found with eGFR.[12] No difference in afamin concentrations between CKD patients and controls was found in an assay-validation study in healthy blood donors and various patient groups, possibly due to low numbers of 15 CKD cases and 22 controls.[29] In the large study at hand in mild to severe CKD patients, we found strong associations between serum afamin and the kidney function parameters eGFR and UACR. Thus, it might be speculated that afamin is linked to a deterioration in glomerular barrier and filtration function.

Remarkably, afamin concentrations were inversely related to adverse kidney outcomes independent of eGFR or UACR, suggesting that it reflects aspects of kidney function other than changes in filtration barrier and total GFR. Because afamin is, aside from its major hepatic expression, also expressed in renal proximal tubular cells (www.proteinatlas.org/ENSG00000079557-AFM), it could potentially serve as a marker of tubular health. Such capacity has previously been observed for uromodulin, which is expressed in the thick ascending limb of the distal tubule and for which a positive relationship between plasma concentrations and kidney health has also been described.[30] The positive relationship between serum afamin and preservation of kidney function was observed despite the fact that afamin is strongly associated with type 2 diabetes.[9] In addition, the relationship with kidney function was found in patients with and without diabetes

in GCKD. This could mean that afamin has different effects on the development of diabetes and corresponding secondary complications on the one hand and kidney diseases on the other hand.

A further possible role of afamin in kidney disease might be its relatively high degree of glycosylation.[4,6] Glycoproteins inherit a role in cellular interaction and signaling cascades, and their increased urinary excretion could be deemed an early sign of renal injury and kidney disease progression. The plasma glycoproteome has been discussed to be a potential predictor of nephropathy in diabetic subjects.[31,32] Causes for a damaged kidney tissue leading directly to diabetic nephropathy might be high glucose and a divergent glycation.[32,33]

In an exploratory analysis we investigated the risk prediction properties of afamin in patients with mild to severe CKD patients. Although we observed that the continuous prospective NRI revealed a significant gain in risk prediction accuracy when baseline serum afamin concentrations were added to the equation by Tangri et al[23] which includes age, sex, eGFR, and UACR or the 6-variable risk equation developed by Zacharias et al[21] including UACR, serum creatinine, serum albumin, hemoglobin, urea, and cystatin C (see Table 5), these results have to be considered with caution because we are lacking an external validation of our findings. This could have resulted in an overestimation of the prognostic value. However, especially the comparison with the model by Zacharias et al[21] underscores the independence of afamin from serum albumin.

In case afamin is indeed a marker of tubular health as discussed here, it would broaden the spectrum of captured relevant pathways for CKD progression as current risk equations as those of Tangri and Zacharias do not contain markers of tubular function. Considering afamin would potentially strongly improve the risk prediction for CKD progression and thereby guide the use of novel treatment opportunities that may retard the progression of CKD and postpone the onset of kidney failure.[3]

The main strengths of our study are (1) the large sample size based on a well-defined, relatively homogenous CKD population with a median follow-up of 6.5 years, (2) a centralized measurement of baseline parameters and adjudication of clinical outcomes, and (3) the measurement of afamin with a well-validated ELISA. Most importantly, prospective studies on the association of afamin and kidney failure outcomes were until recently lacking.

Although the analyses were adjusted for major risk factors and parameters of kidney function, residual confounding cannot be excluded and might be a limitation of the study. Furthermore, it remains to be elucidated whether our findings are also of relevance for non-White ethnicities. Because the GCKD study included mainly CKD patients in stage G3 or A3, the findings might not be transferable to other stages of CKD or to general non-CKD populations.

In conclusion, this large prospective study in patients with mild to severe CKD patients revealed an independent and strong association of serum afamin concentrations with kidney failure independent of kidney function and other known risk factors. Afamin might thus be a promising marker for the identification of CKD patients at high risk for disease progression to kidney failure.

Supplementary Material

Supplementary File (PDF)

Figure S1: Distribution of afamin in the GCKD study based on a histogram plot.

Figure S2: Nonlinear *P* spline for afamin concentrations (per 10 mg/L) on kidney failure in the age-, sex, eGFR, and UACR-adjusted Cox regression model. The dashed lines correspond to 95% confidence bands. The blue line refers to the value of the 25th quantile (60.47 mg/L), the red line to the 50th quantile (72.12 mg/L) and the green line to the 75th quantile (84.36 mg/L).

Item S1: Additional analyses.

Table S1: Association of serum afamin with kidney failure during the prospective 6.5 years follow-up based on subdistribution hazard models.

Table S2: Association of serum afamin with kidney failure during the prospective 6.5 years follow-up, comparing KDIGO stages G1-G3a versus G3b and higher.

Article Information

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Association of Serum Afamin With Kidney Failure in Patients With CKD

Setting & Participants

- German Chronic Kidney Disease (GCKD) study
- Prospective cohort study
- N = 5,041 Caucasian patients with CKD stage G3 or A3
- Afamin: Vitamin E-binding glycoprotein primarily expressed in liver & kidney

Results

At Study Entry

Mean (±SD) afamin concentration: 73±18 mg/L

Follow-up:

6.5 years

Each 10 mg/L higher level of afamin concentration was associated with:

- **2.60 mL/min/1.73 m² higher eGFR** (95% CI 2.30-2.89)
- **5.97 mg/g lower UACR** (95% CI 3.04-8.90)
- **14% lower risk of kidney failure**
  HR: 0.86
  (95% CI: 0.81-0.92, *P*<0.001)

CONCLUSION: Higher serum afamin concentrations appear to be associated with a higher eGFR, less albuminuria, and a lower risk for future kidney failure in patients with CKD.

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